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CHEMICAL BIOLOGICAL CENTER

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ECBC-TR-400

DECON GREEN™
DEVELOPMENT AND CHEMICAL BIOLOGICAL AGENT EFFICACY TESTING

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13. SUPPLEMENTARY NOTES *When this work started, the U.S. Army Edgewood Chemical Biological Center (ECBC) was known as the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC).					
14. ABSTRACT The development of DECON GREEN™ from its inception to the present is described, and efficacy data for VX, GD, TGD, HD, THD, and anthrax are presented. Examples of consumer products containing the identical or similar ingredients of DECON GREEN™ are given. The efficacy data reveals the tremendous decontamination efficacy afforded by a solvent-based, material-penetrating decontaminant. However, materials susceptible to agent absorption and absorption of the decontaminant are apt to suffer deleterious effects – the inevitable price of thorough decontamination.					
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PREFACE

The work described in this report was authorized under Project No. 206023.84BPO. The work was started in March 1997 and completed in May 2003.

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DECON GREENTM

DEVELOPMENT AND CHEMICAL BIOLOGICAL AGENT EFFICACY TESTING

1. INTRODUCTION

In 1997, the late Prof. Russell S. Drago, U. FL, discovered that bicarbonate ion catalyzed the oxidation of sulfides. The exciting implication of this finding was that HD, also a sulfide, should also undergo similar oxidation as a means to decontaminate it. At the time, the perhaps preposterous suggestion that such a simple and environmentally friendly mixture, i.e., essentially the common consumer goods baking soda and hydrogen peroxide, could be used to decontaminate HD seemed too good to be true. Yet, the enormous potential of a decontaminant based on such innocuous ingredients warranted investigation as a means to develop an environmentally acceptable replacement for DS2. Moreover, since hydrogen peroxide is a known biocide, the bicarbonate/peroxide system also promised to simultaneously afford the decontamination of biological agents.

Initial studies conducted by Wagner and Yang at ECBC¹ indeed found that bicarbonate-activated peroxide, in conjunction with t-butanol co-solvent, was effective at selectively oxidizing HD to the non-vesicant sulfoxide, avoiding formation of the vesicant sulfone.² Thus the bicarbonate/peroxide system proved its ability to decontaminate mustard. However, to be broad-spectrum chemical agent decontaminant like DS2, the bicarbonate/peroxide system would have to be effective against nerve agents as well.

Previous studies performed decades ago had shown that basic peroxide is effective against G-type nerve agents.³ And, more recently, Yang et al.⁴ found that basic peroxide was a particularly effective decontaminant for VX as the reaction was selective, avoiding formation of toxic EA-2192. Indeed, follow-on studies of the bicarbonate/peroxide system by Wagner and Yang⁵ found it was effective against GB and VX, although somewhat less reactive for the latter. With these results for the nerve agents and the earlier HD data, Wagner and Yang filed for a patent in 1999, which was awarded in 2001.⁶

Although the bicarbonate/peroxide/alcohol system was effective for GB, VX and HD, it was problematic for three reasons. First of all, it lacked sufficient capacity for VX. Secondly, the rate of the HD reaction was slow compared to the nerve agent reactions, especially at the higher pH required for efficient VX reaction. And finally, alcohol co-solvents, which are objectionable for at least three reasons: (1) alcohols are ineffective for the dissolution of thickened agents and minimally effective at rapidly dissolving HD, (2) environmentally acceptable alcohols, including the favored, edible ethanol, possess unacceptably low flash points, and (3) alcohols lack sufficient detergency to adequately clean and decontaminate dirty and oily surfaces. It was at this time that an early trial formula containing isopropanol, or "rubbing alcohol" (another consumer alcohol possessing a marginally higher flashpoint than ethanol), and Triton[®] X-100 (see below) co-solvents, was tested against DS2 for the decontamination of HD-contaminated CARC panels. Bartram et al.* determined that the decon efficacy of this trial formula greatly exceeded that of DS2, confirming that such a decontaminant had great potential

* Bartram, P. W., Procell, L. R. and Wagner, G. W. U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, unpublished results, 2000.

to be a viable replacement for DS2. Furthermore, spray trials* of the same formula conducted at Porton Down, UK, for TGD-, THD-, and VX-contaminated panels yielded similar decon efficacy for horizontal and vertical panels, contrary to the popular belief that a clinging foam is necessary to provide adequate decon performance on non-horizontal surfaces.

To solve the first problem, Wagner et al.⁷ increased the VX reactive capacity by utilizing carbonate, in addition to bicarbonate, although this resulted in a slight decrease in HD reactivity owing to the higher pH. Additionally, the more soluble potassium salts, rather than sodium salts, were employed to allow higher bicarbonate/carbonate content. The potassium salts offered an additional benefit in that they dissolved much more rapidly than the sodium salts; an important feature to facilitate operational mixing by the end user. Realizing at this stage that VX, GB, and HD could be decontaminated with sufficient capacity using only the environmentally friendly materials bicarbonate (baking soda), carbonate (washing soda), and hydrogen peroxide, Wagner coined the name “DECON GREEN™” in 1999 for the emerging decontaminant.^{7,§} Eventually, bicarbonate would be dropped from the formula entirely as it quickly became apparent that, with the addition of molybdate (see below), only carbonate, in a buffering capacity, was needed.

The second and third problems were solved nearly simultaneously. In fact the problem of slow HD reactivity already had a solution waiting in the wings. In 1998, Prof. Clifford A. Bunton[†] had suggested using molybdate as a peroxide activator for HD oxidation, although the need for an alternative activator that could function at the higher pH needed for the VX reaction did not become apparent until 1999. Wagner et al.⁸ did find that molybdate, as an activator, was about two orders of magnitude more powerful than bicarbonate for the oxidation of HD and, just as important, that molybdate had no deleterious effect on the reaction of VX. At the same time, trial oxidations using molybdate were successfully carried out in microemulsions consisting of propylene carbonate (oil phase) and Triton[®] X-100 (surfactant). Propylene carbonate was chosen as a replacement for the alcohol co-solvent for a variety of reasons: (1) very high flashpoint (132 °C/270 °F, non-flammable), (2) extremely low freezing point (-55 °C/-67 °F), (3) it dissolved HD and thickened agents extremely well, and (4) it is used in cosmetics, including mascara and lip gloss. Triton[®] X-100 was chosen for similar reasons: (1) it is a non-ionic surfactant possessing legendary grease-cutting ability, (2) it has a high flashpoint (>110 °C/>230 °F), and (3) it quickly dissolves HD. Thus, molybdate, propylene carbonate, and Triton[®] X-100 were all simultaneously included into the DECON GREEN™ formula, and Wagner et al.[‡] applied for a second patent in 2002. Subsequent to their inclusion into DECON GREEN™, it was found that molybdate is a source of molybdenum, an essential mineral, and it is found in vitamins and nutrition bars, and Triton[®] X-100 is used in agricultural spraying operations. Thus, the use of molybdate, propylene carbonate and Triton[®] X-100 is in keeping with the use of only environmentally friendly ingredients in DECON GREEN™. Moreover, the end user of DECON GREEN™ is already using and/or ingesting similar if not identical ingredients, which are contained in everyday consumer products shown in Figure 1.

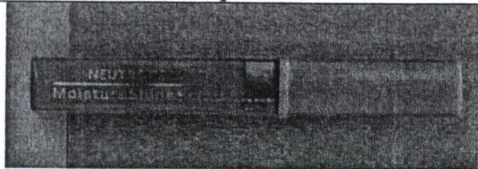
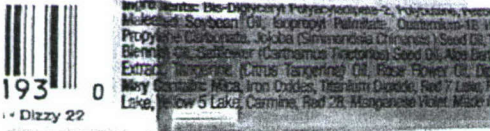

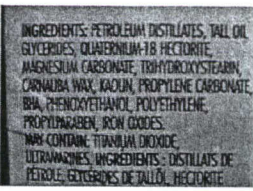

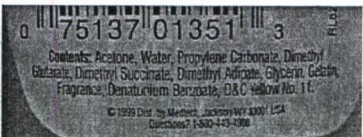
* Govan, N., Ward, A. D., Wagner, G. W. and Procell, L. R. U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, unpublished results, 2000.

§ Trademark applied for.

† Wagner, G. W. and Bunton, C. A. Discussions at 1998 ERDEC Scientific Conference on Chemical and Biological Defense Research, Nov. 17-20, 1998.

‡ Wagner, G. W., Procell, L. R., Yang, Y.-C. and Bunton, C.A., US Patent applied for.

Figure 1. Consumer Products Containing Identical or Similar DECON GREEN™ Ingredients

Propylene Carbonate		
Lip Gloss	Mascara	Nail Polish Remover
  <p>INGREDIENTS: Bis-Diglycerin Polychlorosilates-2, Polyoxane, Penta Methylol Soybean Oil, Isopropyl Palmitate, Quaternium-15, 11 Propylene Carbonate, Jojoba (Simmondsia Chinensis) Seed Oil, Biotin, Biotinyl, Biotinyl (Carnitine Triester) Seed Oil, Aloe Vera Extract, Tangerine (Citrus Tangerine) Oil, Rose Flower Oil, Di Methyl Caprylate, Iron Oxides, Titanium Dioxide, Red 7 Lake, Yellow 5 Lake, Carmine, Red 2B, Manganese Violet, Mica.</p>	  <p>INGREDIENTS: PETROLEUM DISTILLATES, TALL OIL, GLYCERIDES, QUATERNIUM-15, HECTORITE, MAGNESIUM CARBONATE, TRIHYDROXYSTEARIN, CARVALUBA WAX, KAGUIN, PROPYLENE CARBONATE, BHA, PHENOXYETHANOL, POLYETHYLENE, PROPYL PARABEN, RON COODES. MAY CONTAIN: TITANIUM DIOXIDE, ULTRAVIOLET. INGREDIENTS: DISTILLATES DE PETROLE, GLYCERIDES DE TALLOIL, HECTORITE.</p>	  <p>Contents: Acetone, Water, Propylene Carbonate, Dimethyl Glutarate, Dimethyl Succinate, Dimethyl Adipate, Glycerin, Gelatin, Fragrance, Denaturatum Benzoin, D&C Yellow No. 11.</p>

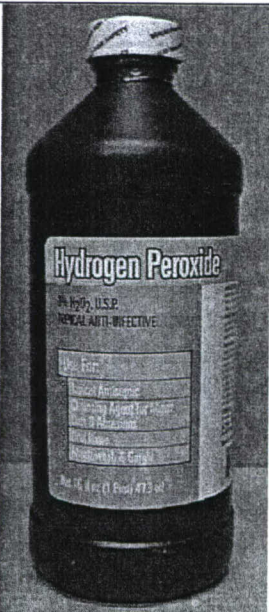


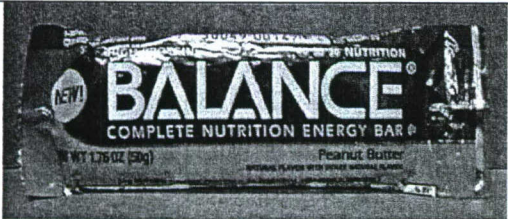
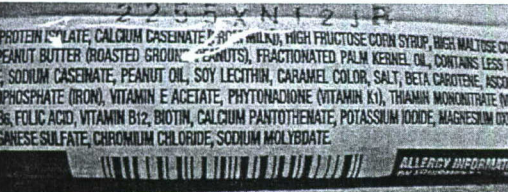

Hydrogen Peroxide (3%)	
	<p>cleanse the mouth.</p> <p>DIRECTIONS: Apply locally to affected areas. To cleanse the mouth, dilute with an equal amount of water, swish around in mouth for at least one minute, then spit out, try to avoid swallowing this product.</p> <p>WARNINGS: FOR EXTERNAL USE. Do not use in eyes or apply over large areas of the body. Do not use on deep or puncture wounds, animal bites or on serious burns. If redness, irritation, swelling or pain persists or increases or if infection occurs discontinue use and consult a physician. Do not use this product for more than 7 days unless directed by a dentist or doctor. KEEP THIS AND ALL DRUGS OUT OF THE REACH OF CHILDREN. In case of accidental ingestion, seek professional assistance or contact a Poison Control Center immediately.</p> <p>STORAGE: Keep bottle tightly closed and at controlled room temperature 59° - 86°F (15° - 30°C). DO NOT SHAKE BOTTLE</p> <p>ACTIVE INGREDIENT: Stabilized Hydrogen Peroxide 3% ALSO CONTAINS: Purified Water.</p> <p>Manufactured by ... TN 37167</p>

Figure 1. Consumer Products Containing Identical or Similar DECON GREEN™ Ingredients
(Continued)

Non-ionic Surfactants	
	<p>Do not add bleach. / NO ANADA blanqueador</p> <p>Dawn contains biodegradable anionic and nonionic surfactants and no phosphate. / Dawn contiene surfactantes biodegradables aniónicos e iónicos y no contiene fosfatos.</p> <p>Keep out of reach of children. If Dawn gets in eyes, rinse thoroughly with water. If swallowed, drink a glass of water to dilute. / Mantenga fuera del alcance de los niños. Si Dawn entra en contacto</p>

Sodium Carbonate ^a	
	<p>CAUTION: Contains Sodium Carbonate. Avoid contact with eyes. If contact is made, flush with water for 15 minutes. If swallowed, give water or milk. Contact a physician.</p> <p>KEEP OUT OF REACH OF CHILDREN</p>

^aHandling and safety precautions similar to potassium carbonate.

Sodium Molybdate*	
 	 <p>g) Riboflavin, Lecithin, Thiamine Mononitrate, Sodium & Potassium Borates, Beta Carotene, Blue 2 Lake, Folic Acid, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Carboxymethylcellulose, Sodium Metavanadate, Sodium Citrate, Phytonadione, Biotin (USP Method 2), Nickelous Sulfate, Stannous Chloride, Ergocalciferol, Cyanocobalamin, Sodium Metasilicate.</p>

*Handling and Safety precautions similar to potassium molybdate.

Having solved the reactivity dilemma for VX and HD, attention turned to the much-anticipated ability of peroxide-based DECON GREEN™ to destroy biological agents. Exceeding all expectations, Rastogi et al.⁹ found that trial DECON GREEN™ formulas exhibited 7-log kills of anthrax spores (*Bacillus anthracis*). Thus, the chosen ingredients of DECON GREEN™ were deemed appropriate for not only the rapid decontamination of chemical agents, but also for bio agents.

In February of 2002, Marine Corps Systems Command (MARCORSYSCOM) issued a call for decontaminants for its Joint Service Family of Decontamination Systems (JSFDS) program. DECON GREEN™, with its excellent decon efficacy performance for chemical and biological decontamination, was judged sufficiently mature to propose the decontaminant to the program. However, a snag came up in reviewing the requirements: the need to ship decontaminants by air. Up to this point, the strength of hydrogen peroxide used in DECON GREEN™ had been a 50 wt% aqueous solution (a common commercial grade) which, besides containing more active peroxide and less inactive, ineffective water, affords better decon efficacy than 35 wt% aqueous peroxide (a second, common commercial grade). In addition to providing better decon efficacy, 50 wt% peroxide also has a lower freezing point than 35 wt% (-50 °C/-58 °F versus -33 °C/-27 °F), which may be an important consideration for low temperature decontamination. But 50 wt% cannot be transported by air whereas 35 wt% is permissible.* Thus, for this reason, a switch was made to 35 wt% peroxide in the DECON GREEN™ formula.

In April 2002, DECON GREEN™ was selected to participate in the CASPOD (Contamination Avoidance at Sea Ports of Debarkation) program, which involved primarily wide area decontamination and extremely large volumes of decontaminant. Thus a final, but minor change to the formula was necessitated to facilitate handling and mixing DECON GREEN™ on a large scale. The solid molybdate and carbonate activators were pre-dissolved in a minimum amount of water, conveniently converting the once liquid-liquid-solid formula to a three-liquid formula. However, this limited storage and use of the three-liquid formula to -25 °C/-13 °F (the freezing point of the aqueous molybdate/carbonate solution). (Previously, when employing the activators as solids, the freezing point of the 35 wt% hydrogen peroxide, -33 °C/-27 °F, was the limiting component for low temperature use.) Yet, raising the low temperature limit was deemed an acceptable penalty in view of the greater ease of handling and mixing liquids, versus solids, in large quantities.

To support participation in the CASPOD program, ECBC partnered with Steris Corporation to produce, package, and ship the large quantities of DECON GREEN™ needed for testing. It was also decided during this period to seek registration of the DECON GREEN trademark; henceforth referred to as DECON GREEN™.

The data presented below is for the first DECON GREEN™ “Classic” formula, which was proposed to the JSFDS program and used in initial CASPOD testing in 2003. A “New” DECON GREEN™ formula is currently being developed to address some of the

* Transport of 35 wt% hydrogen peroxide is limited to 5 L size containers for commercial air cargo. There is no size limitation for military air transport.

shortcomings of the “Classic” formula found during CASPOD testing and as a result of other emerging requirements. “New” DECON GREENTM test data will be presented in a future report.

2. EXPERIMENTAL PROCEDURES

2.1 Reactor Tests.

Reactions were carried out in glass-jacketed reactors fitted with mechanical stirrers at 25 °C. Reactions were simultaneously run in triplicate in three identical reactors. In a typical run, 50 mL of decontaminant was added to each of the three reactors, the stirrers were started, and 1 mL of agent was added to each of the three reactors. At desired times, 59 µL samples were removed from the reactor and quenched with 1 mL of 0.2 M sodium sulfite and 0.2 M sodium carbonate, and extracted with 2 mL chloroform. The chloroform layer was analyzed for residual agent by GC-AED.

2.2 Anthrax Tests.

Test was conducted using avirulent *Bacillus anthracis* (NNRA1; plasmid-free) as previously described.⁹ A 0.1 mL water suspension containing 7×10^7 spores was spotted on a sterile glass slide and left to dry overnight in a bio-hood. The dried spores were washed/recovered using two-0.5 mL portions of decontaminant. The slide was abraded with a pipette tip to help loosen the spores. Within 10 min, serial dilutions were performed from the 1 mL decontaminant solution containing the spores. From each serial dilution, 100 µL aliquots were plated on nutrient broth-agar plates. The plates were incubated at 37 °C, and observed for growth at 24, 48, and 72 hr.

2.3 Panel Tests.

CARC-painted panels, 2 in. in diameter, were employed. Six replicates were used for each decontaminant. The panels were contaminated with 2 µL drops of agent to yield a contamination density of 10 g/m². Droplets were spread around with a piece of parafilm to form a thin, uniform film of the agent on the panel. The panels were covered to prevent excessive evaporation in the fume hood and allowed to stand for 1 hr. A volume of 1 mL decontaminant (1:50 agent: decontaminant ratio) was applied to the panels, evenly distributed with the pipet tip, and allowed to stand covered for 15 min. Excess decontaminant was then poured off, and the panels were rinsed with two 20 mL portions of water. The panels were allowed to air dry in a vertical position in the fume hood for 2 min. After drying, contact tests were conducted by placing the following, in order, on top of the panels for 15 min: 2 in. latex disk; 2 in. aluminum foil disk; 2 in. diameter 1 kg weight. The latex disk was removed, and the panel and latex disk were extracted in 20 mL chloroform (containing 1 mL/L thiolane to quench remaining peroxide) for 1 hr. Solutions were analyzed by GC/FID to determine the amounts of agent recovered.

3. RESULTS AND DISCUSSION

3.1 Reactor Tests.

Table 1 gives stirred reactor data for DECON GREEN™ Classic with conventional agents HD, VX, and GD. All of the agents are rapidly decontaminated with half-lives of 1 to 3 min to non-detectable levels, and exhibit the desired capacity of 1:50 agent to decontaminant.

Table 1. Reactor Data for DECON GREEN™ Classic*

Time	% HD			% VX			% GD		
10 min	0.3	1.7	4.5	1.8	3.6	3.1	0.0	0.2	0.0
20	0.0	0.1	0.1	0.0	0.1	0.0	—	0.0	—
30	—	0.1	0.1	—	0.0	—		—	
40		0.0	0.0		—				
50		—	—						
t _{1/2} (min)	0.95	1.71	2.02	2.18	2.38	2.61	1.33	1.36	1.51

*1 mL agent in 50 mL decon, 25 °C. Triplicate runs in stirred reactors. Results expressed as % agent remaining.

3.2 Anthrax Tests.

Table 2 shows that a 7-log kill of anthrax spores was achieved within 15 min.

Table 2. DECON GREEN™ Classic Anthrax Spore Decontamination*

Decon	Challenge	CFU Recovered	Log Kill
DECON GREEN™ Classic	7×10^7	< 10 (no viable spores detected)	> 7-log

*15 min decontamination time.

3.3 Panel Tests.

Table 3 gives results for DECON GREEN™ Classic decontamination of CARC panels contaminated with HD, THD, TGD, and VX. Results for DS2 and DF200 are also given for comparison. For these tests, DF200 was applied as a liquid.

Table 3. Decontamination of HD, THD, TGD, and VX on CARC Panels^a:
DECON GREENTM Classic Versus DS2 and DF200^b

	HD		THD		TGD		VX	
Decon	Cont.	Resid.	Cont.	Resid.	Cont.	Resid.	Cont.	Resid.
DECON GREEN ^{TMa}	5.7	17.5	1.8	6.1	3.0	12.9	3.3	39.5
DS2	5.2	54.1	2.3	7.2	3.0	23.9	3.6	77.0
DF200	30.7	207.5	454.9	101.4	4.6	26.0	11.8	110.5

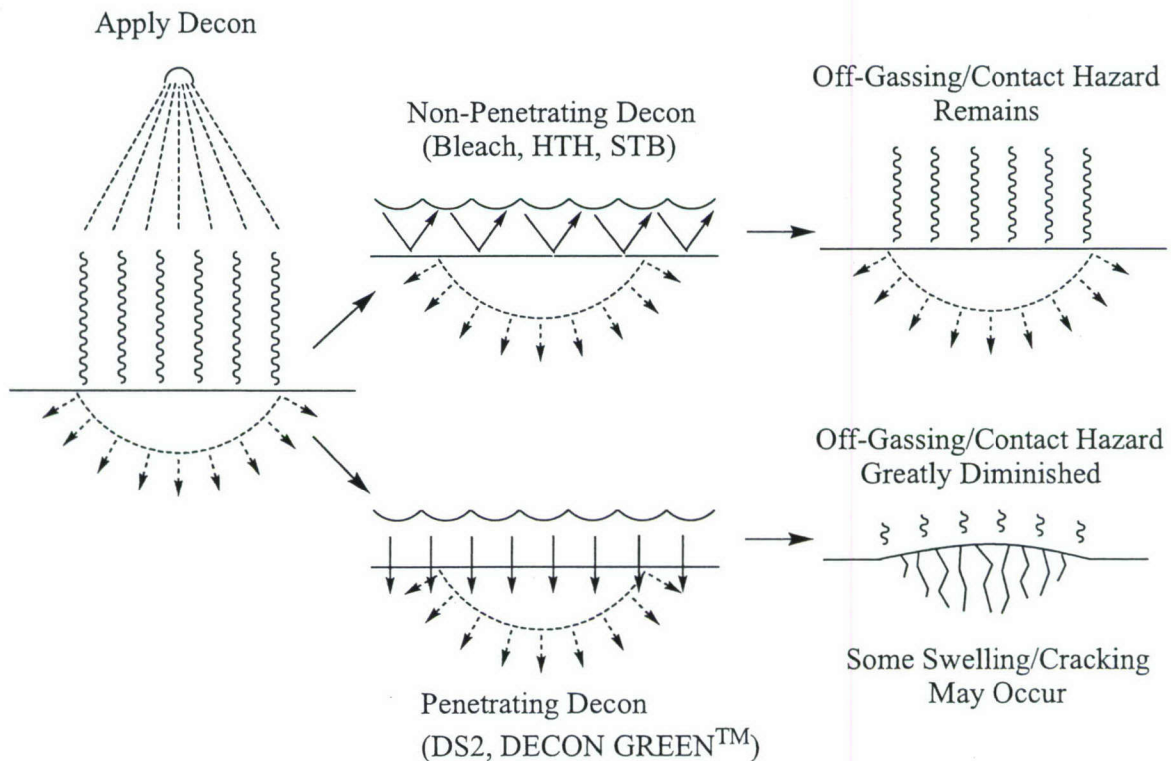
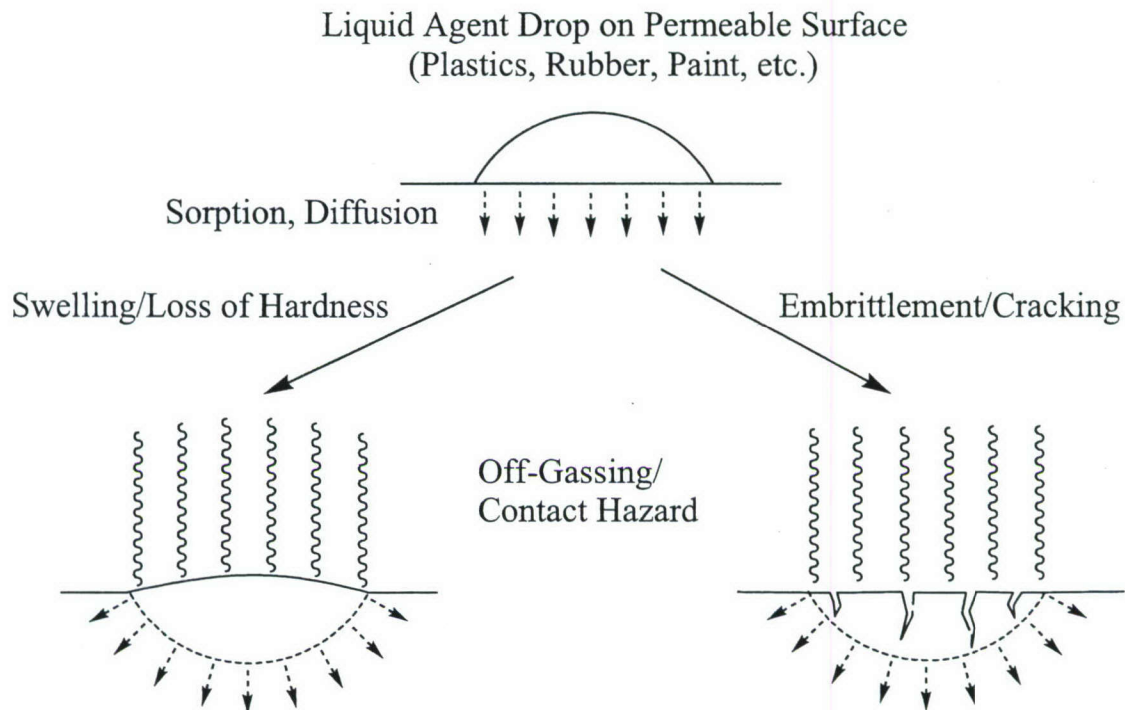
^aInitial contamination level 10 g/m². Agent dwell time 1 hr 15 min decontamination time. Contact hazard and residual agent expressed in micrograms/square centimeter. Average of six replicates reported.

^bEnvirofoam Technologies.

It is readily apparent that DECON GREENTM is comparable to DS2 at reducing the contact hazard, but exceeds DS2 at removing the residual agent hazard. Except for TGD, DECON GREENTM and DS2 far exceed the decontamination efficacy of DF200. A reasonable explanation for these observations is that DECON GREENTM and DS2 possess a high content of penetrating organic solvent that can access and react with agent absorbed within susceptible materials such as plastic, rubber, and painted surfaces. However, in the act of penetrating these susceptible materials to decontaminate absorbed agent, some unavoidable damage may occur such as softening and cracking. These processes are depicted in Figures 2 and 3.

As shown in Figure 2, CW Agents, just like any other organic solvent, can penetrate, cause swelling, cracking, and embrittlement of susceptible materials. For example, HD is known to dissolve acrylic and polystyrene; penetrate silicone and polyurethane; cleave, crack and embrittle polycarbonate; and soften polyvinylchloride (PVC).^{10,11} VX also dissolves acrylic, penetrates silicone, and distorts and cracks polyurethane.¹⁰ Thus, if these materials came into contact with HD and/or VX during a CW attack, they would be expected to suffer some damage prior to any decontamination effort. Moreover, once absorbed into the material, HD and VX would off-gas and/or be contact hazards for an extended period of time.

In Figure 3, the difference between penetrating decontaminants, e.g., DS2 and DECON GREENTM, versus non-penetrating decontaminants such as HTH, STB, and bleach is depicted. The non-penetrating decontaminants merely react with and remove agent from the surface, leaving absorbed agent to become a contact and off-gassing hazard. Conversely, the penetrating decontaminants absorb into the material to decontaminate the absorbed agent, thus effecting substantial reductions in off-gassing and contact hazards. Of course, penetration of the material by the decontaminant will result in additional softening/cracking/embrittlement beyond that caused by the agent itself. However, this additional damage may be an acceptable trade-off when the costs of prolonged off-gassing and contact hazard on soldier health and the continued burden of having to wear substantial protective gear are considered.



As an illustration of the effect of penetrating versus non-penetrating decontamination, panel test results for the decontamination of HD on acrylic paint panels are shown in Table 4. As discussed above, HD softens or penetrates acrylic plastics;¹⁰ thus, it was anticipated that HD would substantially absorb into acrylic paint. The results in Table 4 show that this is indeed the case. Aqueous HTH (10%), for example, is able to react with HD still residing on the surface of the alkyd paint, thereby reducing the contact hazard to a level substantially below that of plain water. Water, on the other hand, does not dissolve HD very well, and thus leaves most of the surface HD behind. However, HTH does not penetrate the alkyd paint to react with absorbed HD (Figure 3), so a substantial fraction of the HD remains as a residual hazard. Indeed HTH is only minimally more effective than water at reducing the residual hazard. On the other hand, DECON GREENTM, which is known to penetrate and soften alkyd paint (as well as CARC),¹² is most effective at penetrating the alkyd paint to react with absorbed HD (Figure 3), substantially decreasing the contact and residual hazards. Moreover, considering the amount of the original agent actually accessed and decontaminated, DECON GREENTM is able to decontaminate 94.4%; whereas, HTH and water only decontaminate 59.4 and 34.5%, respectively.

Table 4. Decontamination of HD on Alkyd-Painted Panels^a

Decontaminant	Cont.	Resid.	Total	% ^b
DECON GREEN TM	18.2	37.8	56.0	94.4
HTH (10%)	72.1	334.3	406.4	59.4
Water	196.6	458.8	655.4	34.5

^aInitial contamination level 10 g/m² (1000 µg/cm²). Agent dwell time 1 hr 15 min decontamination time. Contact hazard and residual agent expressed in micrograms/square centimeter. Average of six replicates reported.

^bPercent of original agent decontaminated.

4. CONCLUSIONS

DECON GREENTM is a broad-spectrum decontaminant, effective against chemical agents VX, GD, HD, and biological agents such as anthrax. DECON GREENTM is more effective than DS2 at decontaminating VX, HD, THD, and TGD on CARC painted panels. DECON GREENTM and DS2 are dramatically more effective than DF200 at decontaminating VX, HD, and THD on CARC painted panels; whereas, DF200 is only slightly less-effective for decontaminating TGD on CARC painted panels.

DECON GREEN'sTM superior performance is predicated on its ability to penetrate and absorb into materials, which are likewise susceptible to agent penetration and sorption. Unfortunately, when materials are penetrated by agent and/or decontaminants, they suffer softening, cracking, and embrittlement. However, these side effects are unavoidable if quick, thorough decontamination is truly desired. Such thorough decontamination, provided by penetrating decontaminants, protects the health of soldiers and facilitates their use of decontaminated equipment while wearing a minimum amount of protective gear.

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